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Single-Cell Voltage Measurements with a Set of Nanoprobes

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Noninvasive single cell electrical measurements using carbon nanotubes as electrodes are reported here. The device consists of four nanotubes deposited in the corner of a 2 micron square. Using flow, single cells are places on top of the electrodes. Two of the probes are used to apply voltage pulses to the cell and the other two are used to measure the response as a function of time. As a control, measurements of water, cell medium, cells and biomolecules have been made with metallic plates, defined by 60nm holes in a 75nm insulating film. For proof of principle, yeast cells suspended in HEPES are measured. The results show that the nanotubes allow a contact with the ionic environment 100 times better than the metallic plates. The nanotubes also show a different response when the cell is nearby or touching a cell. Since the nanotubes are 1.2 nm in diameter, comparable in size with membrane proteins, we plan to use the nanotube array to perform some of the functions of patch clamps but with less perturbation to the cells due to the small dimension of carbon nanotubes.

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A Comprehensive Live Cell Screening Approach for Developing Improved Microbial Rhodopsin-Based Voltage Biosensors

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Fluorescence imaging of neuron activity would be greatly facilitated by the availability of brightly fluorescent genetically encoded voltage indicators with large and fast responses to membrane potential changes. The Cohen group has recently described a new class of genetically encoded voltage indicators based on the microbial rhodopsin protein archaerhodopsin-3 (Arch) with excellent properties. However, this class of voltage indicators suffers from poor quantum efficiency that limits the range of potential applications. To address this shortcoming, we developed a strategy for directed evolution for brighter Arch-based voltage indicators. Briefly, a random genetic library of Arch is fused to the N-terminus of the fluorescent protein mOrange2 and is expressed in E. coli. Hundreds of colonies on a Petri dish are ratiometrically imaged (i.e., the ratio of Arch to mOrange2 fluorescence) using a custom imaging system. The colonies with the highest ratio are picked and cultured in liquid media. The fluorescence of the overnight cultures is then recorded with a microplate reader. The brightest Arch variants are expressed in Hela cells and their voltage sensitivities are evaluated in a fluorescent microscope by electric field stimulation. The brightest functional variants are then used as the library template for the next round of directed evolution. Several iterative rounds of this screening procedure combined with further screening of site-directed mutagenesis libraries resulted in identification of our current best voltage-sensitive Arch (vArch) variant, vArch1.0. vArch1.0 is voltage sensitive, fast and several-fold brighter than wild-type Arch in mammalian cells. Unlike Arch, vArch1.0 doesn't generate photo-induced current that perturbs membrane potential of cells during imaging. vArch1.0 is capable of resolving electrically triggered action potentials in neurons in single trials with optical signal-to-noise ratio >30 and is a promising tool for optical interrogation of complex neural circuit.

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Biosensing Properties of Au Loaded Mesoporous Silica Nanospheres Coated with Lipid Bilayers

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We have developed a simple synthetic route to achieve the synthesis of gold loaded radial mesoporous silica nanoparticles (Au-MsNPs). These nanoparticles were synthesized in a one step procedure fully compatible with basic conditions required for the preparation of monodispersed nanospheres. These Au-MsNPs were characterized by transmission and scanning electron microscopy, Energy Dispersive X-ray analysis and N2 adsorption. Metallic Au-nanoparticles embedded in pore channels were responsible for plasmonic activity. Au-MsNPs were then coated with phospholipid bilayers in order to design a bio-functional device with plasmonic properties for biosensing. The supported lipid bilayers were obtained after incubation of Au-MsNPs particles with different lipid vesicles. The coating efficiency was investigated by zeta potential, agarose gel electrophoresis and cryo-transmission electron microscopy. Different model systems have been investigated: direct adsorption of bovine serum albumin or molecular recognition events between a biotin receptor (integrated in the supported lipid bilayer) and avidin. Overall the results demonstrate the plasmonic sensitivity of the bare or the coated lipid bilayer Au-MsNPs (Veneziano et al. 2012).

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Nanoparticle-Membrane Interactions Studied with Lipid Bilayer Arrays

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Nanoparticles (NPs) have been explored for use in biomedical applications including gene and drug delivery, and recent studies have indicated that they are also cytotoxic and have significant interaction with lipid bilayer membranes. An understanding of the interactions between NPs and the cell membrane systems will help aid the design of NPs for beneficial applications and evaluate the cytotoxicity of environmental NPs. To study these interactions, we measured amine- and carboxyl-modified polystyrene NPs in arrays of 24-32 artificial lipid bilayers. The throughput possible with simultaneous electrical measurement of the bilayer arrays enabled study of NP/bilayer interactions while varying lipid composition, ionic strength, pH, voltage, and particle type. Our studies showed that amine-modified NPs interacted with bilayers whereas carboxyl-modified NPs did not. The interaction of the amine-modified NPs could be altered by changing the charge density on the bilayers, the ionic strength and pH.

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Liquid-Crystal-Based Biosensor without Alignment Substrate

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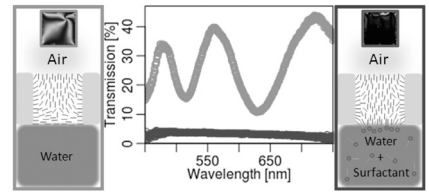
Liquid-crystal-based biosensors, based on a design by N. L. Abbott [1,2], are drawing increasing attention because of their dramatic optical response to minute changes at their surface, due, for example, to absorption of biomolecules [see figure.] In this work we simplify and improve the precision and reproducibility of such a biosensor. First, we show that the sensor may be used without separately prepared aligning substrate, which makes measurements more reproducible. Second, analyze the birefringence of the sensing liquid crystal with a spectrophotometer, which makes measurements more precise. Third, we observe the optical response and measure spectral output from every individual cell to account for distribution of liquid crystal thickness in the TEM grid, which makes measurement and analysis more consistent.



We further study the response of the sensor both on an aqueous solution/air surface and immersed in the solution. These improvements will lead to building less expensive, simpler in operation, and more reliable liquid-crystal-based biosensors.

[1] J.M. Brake, M.K. Daschner, Y.-Y. Luk, and N.L. Abbott, Science (New York, N.Y.) 302, 2094 (2003).
[2] W. Iglesias, N.L. Abbott, E.K. Mann, and A. Jákli, ACS Applied Materials & Interfaces 4, 6884 (2012).

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Radio-Frequency Tank Circuit for DNA Sequencing

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DNA sequencing by now is a ubiquitous technique in biology and medicine. Nanopore sequencers turn out to be a promising approach for high-speed sequencing of long DNA-strands while cutting costs by many orders of magnitude.

We present a resonant RLC-circuit operating at radio frequency (Bhat, Soft Nanoscience Letters (2013)). DNA is driven through a nanopore which causes a shift in the resonance of the circuit. The nanopore is embedded within a resonant CPW structure, a so-called tank circuit that enables the measurement of extremely small capacitance changes caused by the transition of the macromolecule through the pore. In contrast to existing nanopore-sequencing technologies, such a design does not rely on a molecular motor to slow down the translocating DNA. Furthermore, the presented method is not limited by the length or a specific design of the pore, allowing a plethora of pore modifications and designs to be used.

Finite element simulation techniques provide an insight into the behavior of different tank-circuit geometries. We present several studies on how the circuit geometry can be modified to increase the sensitivity. Additionally, different concepts for amplification of the atto-Farad signal levels are discussed (Ramachandran et al., Applied Physics Letter 99 (2011)), a degree of sensitivity